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File: USPT

Feb 20, 2001

DOCUMENT-IDENTIFIER: US 6190889 B1

TITLE: Methods for removing primer sequences and blocking restriction
endonuclease recognition domains

DEPR:

In each cycle, adaptor ligation to one end of the DNA segment is followed by class-IIS restriction endonuclease cutting. The recognition domain of the class-IS restriction endonuclease is encoded by the ligated adaptor, allowing restriction endonuclease digestion to trim the DNA segment, generating a new overhang sequence. One or both strands of an adaptor can be ligated, or one or both ends of a single-strand hairpin adaptor can be ligated. Also, one strand of an adaptor can be ligated followed by hybridization, without ligation of the complementary strand, to generate a double-stranded recognition domain. Iterative cycles generate a series of single-strand overhangs, each constituting a DNA template. The single-stranded overhangs are separated by fixed intervals that are limited by the distance between the recognition domain and the cut site in the cleavage domain for the class-IIS restriction endonuclease encoded by the ligated adaptor. This method exploits the separation of the cleavage domain and the recognition domain of class-IIS restriction endonucleases by allowing the sequencing in strides limited only by the distance between the recognition domain and the cleavage domain cut sites, distinguishing it from other iterative approaches. Since each DNA template is a short single-stranded region attached to double-stranded DNA, these single-strands have little opportunity to form secondary structures, providing a considerable advantage over competing methods.